EXPERIMENTAL STUDIES ON THE RELATION OF THE PITUITARY BODY TO RENAL FUNCTION.

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(Received for publication, September 29, 1916.)

INTRODUCTION.

In recent papers I have dealt with the clinical aspects of the relation between the pituitary body and the kidneys, especially with regard to the etiology and pathology of diabetes insipidus. I have come to the conclusion that the pituitary body, as shown by its extracts, exerts a constant, physiological influence on the functional activity of the kidneys in human beings. This action consists in a checking of the flow of urine,—an antidiuretic effect which is most marked when the diuresis is high. I have also reported a case of diabetes insipidus in which organotherapy with the posterior lobe of the pituitary body has been successfully carried out for a period of about 2 years.

This field for investigation is a comparatively new one, and the subject is of great importance not only from a physiological point of view, but from the promise it gives of an improved therapy. During the past year I have investigated this question experimentally.

HISTORICAL.

That there is some connection between the pituitary body and renal activity was first shown in 1901 by Magnus and Schäfer. They found a pronounced increase in the flow of urine in dogs, cats, monkeys, and rats, following the injections of pituitary extracts, and concluded that this influence was exerted directly on the epithelial elements of the kidneys. The same results were obtained by Schäfer and Herring in 1906, though their records show that the influence is far from constant.

Houghton and Merrill, in 1908, found that the increase in flow of urine in the dog lasted on an average of 19 minutes, and that the effect was dependent on the increase in the general blood pressure. A year later Halliburton, Candler,

and Sikes claimed that the extracts caused an increased output in cats, and in 1910 Ott and Scott came to the same conclusion.

Pentimalli and Quercia, in 1912, from experiments on the isolated kidney of the rabbit, showed that hypophysial extracts diminished the flow as well from the ureter as from the renal vein.

King and Stoland, in 1913, working on etherized dogs, were able to demonstrate an increase in the general blood pressure for 10 minutes, accompanied by swelling of the kidney with subsequent temporary increased flow of urine lasting for 20 minutes. They hold the view that the increased flow is due to renal vasodilatation. In the same year Gabriels, working on the isolated kidney of the dog, found increased flow without vasodilatation. On the other hand, Beco and Plumier, in 1913, noted a transient diminution of flow. In the same year Iscovesco maintained that a lipoid from the anterior lobe had a diuretic action both in rabbits and man.

In 1914, Römer reported that the flow was decreased in rabbits and cats, catheterized every hour.

Hoskins and Means, in 1912, concluded that the diuretic effect found in dogs under narcosis was not due to changes in blood pressure.

Herring, in 1913 and 1914, stated that the diuretic effect of the posterior lobe extracts could be demonstrated in all species of animals, and that extracts from the anterior lobe and the pars intermedia did not possess this power.

Cow, in 1914, found that pituitrin had a diuretic action of 15 minutes' duration in cats.

In 1916, von Meyenburg showed that the injection of extracts caused a decreased flow in rabbits of 8 to 10 hours' duration.

Shamoff, in 1916, found that electric stimulation of the superior cervical ganglion produced increased flow due to excitation of the pituitary body, but his results were inconstant.

The results obtained from observations in man are almost as contradictory.

De Cyon, in 1910, mentions that he had seen diuretic effects from feeding dry pituitary preparations.

Falta, Newburgh, and Nobel, in 1911, found increased diuresis a common result from subcutaneous injections of pituitrin.

Von den Velden, in 1913, was able to check the diuresis in two normal men by hypophysin, and in the same year Frey and Kumpiess confirmed this result by injections of pituglandol. 2 years later von Konschegg and Schuster obtained the same result in four normal subjects. Motzfeldt, in 1914, also found that injections of pituglandol checked the diuresis in a series of fifteen relatively healthy patients, while the anterior lobe extracts did not possess this property.

During the past 3 years a number of cases of diabetes insipidus have been reported in which pituitary extracts have checked diuresis to a considerable extent.

As will be seen, there has been great confusion concerning this question, and this is due chiefly to the fact that results of the experiments in the physiological laboratories have been too readily accepted by clinicians. As a matter of fact, every text-book dealing with this matter states as an ascertained fact that extracts from the posterior lobe are diuretics.

At this point it should be emphasized that the term diuresis is used with different meanings. Some authors use the word to indicate the total 24 hour amount of urine, without regard to the quantity, while others define the term as increase of the quantity of urine.

The confusion is due chiefly to the methods employed. Schäfer carried out his original experiments on anesthetized animals with the drop-recording method, and the observation time was generally very short. The urine was collected from a cannula inserted directly into the bladder. Under these circumstances it cannot be excluded that the increased number of drops may be due, at least partly, to the tonic action on the bladder, which is a constant effect of pituitary injections. There are, moreover, serious objections to operations on the bladder or ureter, as reflex action may interfere considerably with the normal conditions. Furthermore, as we do not know the influence of narcosis on the vegetative nervous system, work on anesthetized animals introduces an uncontrollable source of error.

Most of the subsequent observations have been made by Schäfer's original method, with the same errors and the same misinterpretation of the results. The results, however, have been notably inconstant in the reports of all. Schäfer and Herring, for instance, in 19 experiments on dogs, observed a diuretic effect in 12, and in 7 a diminution of flow. Moreover, most of the work has been carried out with intravenous injections which usually influence the blood pressure considerably.

Method.

The chief aim in finding a suitable method has been to avoid the sources of error mentioned above, and to work as closely as possible under physiological conditions. The work was started on dogs, but experience soon taught that rabbits were more suitable for this

purpose and the majority of the experiments, therefore, have been carried out on these animals.

Having made sure that the 24 hour amount of urine was diminished with three pituitrin injections given at 8 hour intervals, the half hourly output was followed. The animals were placed on the table on their backs and catheterized every half hour. Catheterization of male rabbits is easily done by a soft rubber catheter, and consequently the experiments were carried out on males exclusively.

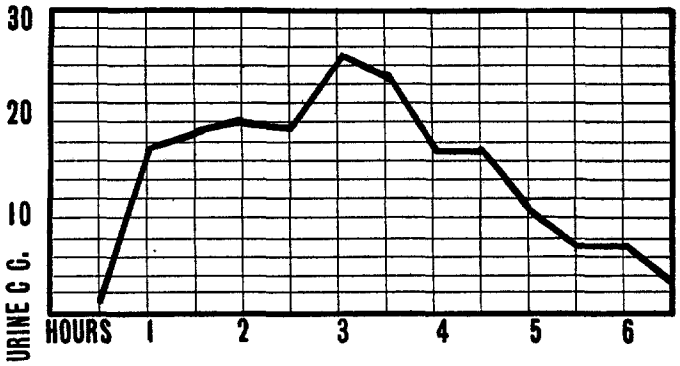
The experience of previous investigators has shown that the position on the back does not interfere with the normal flow of urine and the animals keep perfectly still without anesthesia. Psychic influences, therefore, can be ruled out.

In order to obtain a more marked reaction, I subsequently produced an artificial polyuria. For this purpose 150 cc. and later 200 cc. of water (about 37°C.) were introduced by stomach tube immediately before the animals were placed on the table. This amount is approximately the maximum which the stomach of a medium sized rabbit can hold. This method is much the best way of giving water, the subcutaneous or intravenous administration being less reliable and often unsatisfactory. Wherever the term "artificial polyuria" is used in the legends of the charts, it is to be understood that the polyuria is produced in the way described above.

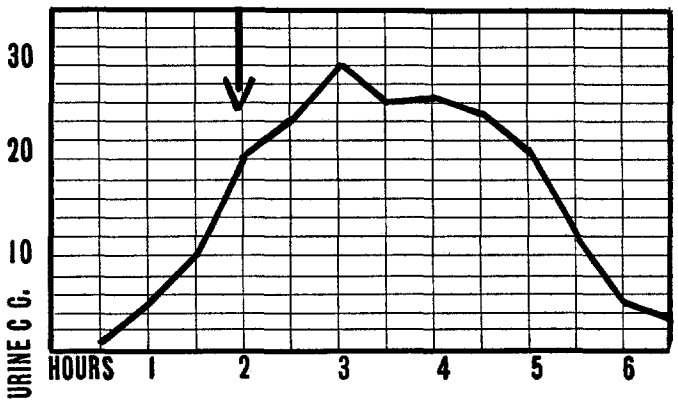
The degree of polyuria is dependent on many factors, particularly, however, on the water content of the tissues, and since this is controlled chiefly by the food, it is of importance to avoid a dry diet and feed the animals mainly on fresh vegetables. Rabbits have occasionally been used several times, as often as every other day, and this is of value because after introduction of water once or twice, the polyuria starts more readily and reaches higher degrees than on the first occasion. The half hourly amounts of urine, obtained in this way, have been plotted as curves, and the resultant polyuria has served as an excellent measure in testing the antidiuretic effect. Only in exceptional instances has the polyuria been delayed for several hours, and, when first started, spontaneous depressions of the output have never been observed.

The polyuria curve is not uniform, and the total amount of urine

returned is subject to great variation. Usually, however, the polyuria starts in 1 to 2 hours and is finished within 4 to 5 hours from the onset (Text-figs. 1 and 2). In estimating the antidiuretic effect, it is, therefore, the relative values which are of chief importance.



TEXT-FIG. 1. Rabbit, weight 2,100 gm. Artificial polyuria. (Water given at the beginning of the observation is indicated by zero.)



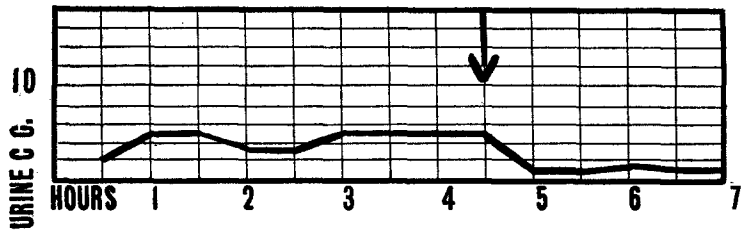
TEXT-FIG. 2. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates 0.7 per cent saline solution, 1 cc. intravenously (as control).

The pituitary extracts employed have been the usual commercial preparations: Pituitrin (Parke, Davis and Company), Pituitary Liquid (Armour and Company), Pituitary Extract (H. K. Mulford Company), Pituglandol (Hoffmann La Roche), Hypophysin (Farbwerke-Hoechst Company), and Extract Hypophysis (Schering and Glatz).

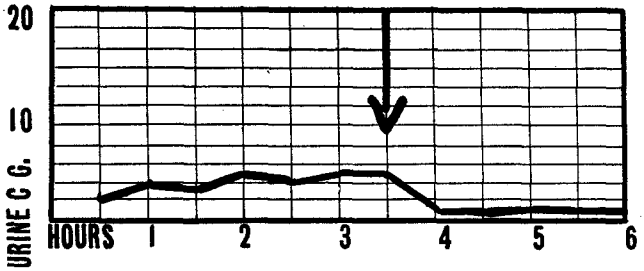
The majority of the experiments have been done without blood pressure registration, and oncometer determinations have not been employed.

Effect of Pituitary Extracts.

The normal half hourly output of urine in rabbits is subject to considerable variation and there is no dependable normal. But even under these circumstances the antidiuretic effect (Text-figs. 3 and 4) of hypophysial extracts is plainly shown (11 observations). It may



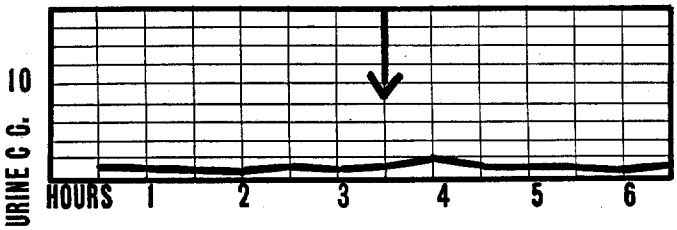
TEXT-FIG. 3. Rabbit, weight 1,400 gm. Normal output of urine. The arrow indicates pituitrin, 1 cc. subcutaneously.



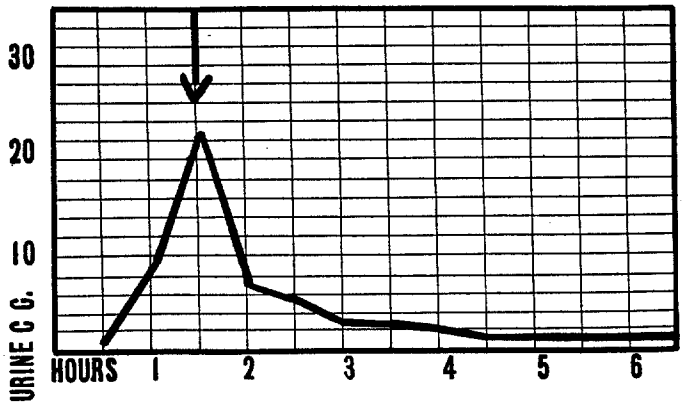
TEXT FIG. 4. Rabbit, weight 1,500 gm. Normal output of urine. The arrow indicates pituitrin, dilution 1: 10, 1 cc. intravenously.

be noted also that the subcutaneous and intravenous injections of the extracts act in the same way, and that when the output is very low (Text-fig. 5) there is no effect to be seen from the injection. This seems to indicate that there is a certain lower limit beyond which the output cannot be depressed. The effect of the extracts on artificial polyuria, however, is far more pronounced (Text-figs. 6 and 7).

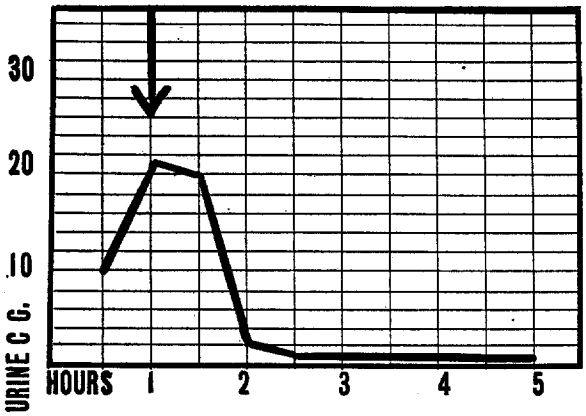
Pituitrin diluted 1: 50, subcutaneously injected in 1 cc. doses, evidently has power to check the output for approximately 5 to 6



TEXT-FIG. 5. Rabbit, weight 1,200 gm. Normal output of urine. The arrow indicates pituitrin, dilution 1: 10, 1 cc. subcutaneously.

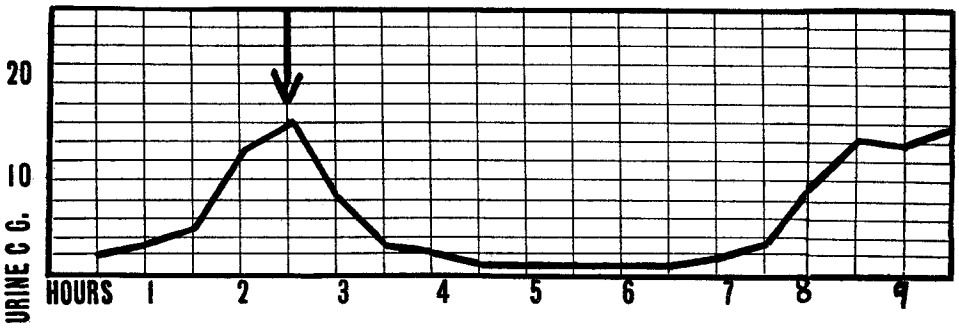


TEXT-FIG. 6. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates pituitary liquid, 1 cc. subcutaneously.

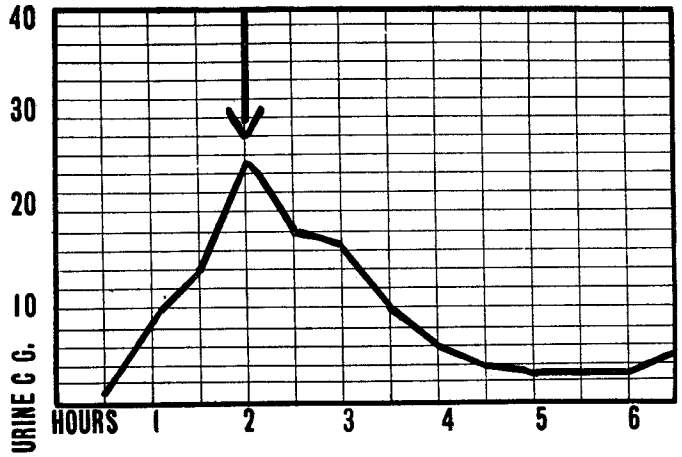


TEXT-FIG. 7. Rabbit, weight 2,400 gm. Artificial polyuria. The arrow indicates pituitary liquid, dilution 1: 10, 1 cc. subcutaneously.

hours (Text-fig. 8). Moreover, it is to be noted (Text-fig. 7) that the influence at times is little marked during the first 30 minutes, and generally reaches its maximum about 2 hours after the injection. This antidiuretic influence may be regarded as constant, for I have



TEXT-FIG. 8. Rabbit, weight 1,600 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 50, 1 cc. subcutaneously.



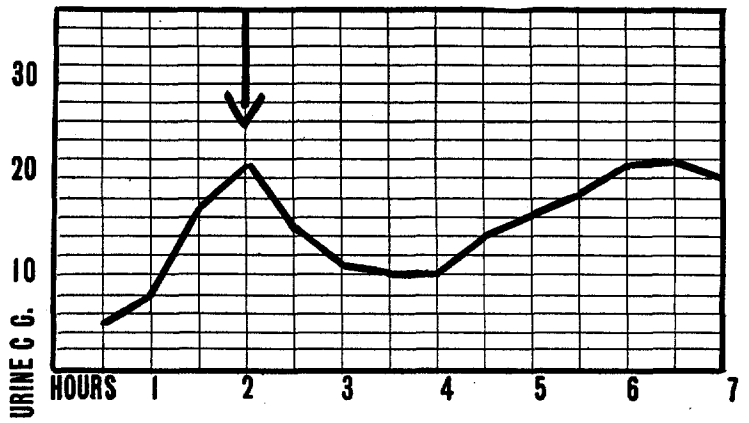
TEXT-FIG. 9. Rabbit, weight 1,400 gm. Artificial polyuria. The arrow indicates pituitrin "400%," 3 cc. by mouth.

seen no exceptions to this rule. The different pituitary extracts have all acted in the same way.

When the urinary output is depressed, the concentration changes in correspondence with the quantity excreted, from colorless to yellowish brown.

The preparations have not been compared as to limit of activity. Most of the work has been done with pituitrin, and the smallest active dose of this extract depends upon its method of administration.

Orally.—(9 observations.) Here there is a slow onset of the renal depression reaching its maximum in about 2 to 3 hours (Text-fig. 9[1]). The lower limit of activity for administration by mouth is apparently 1 cc. of pituitrin (Text-fig. 10).

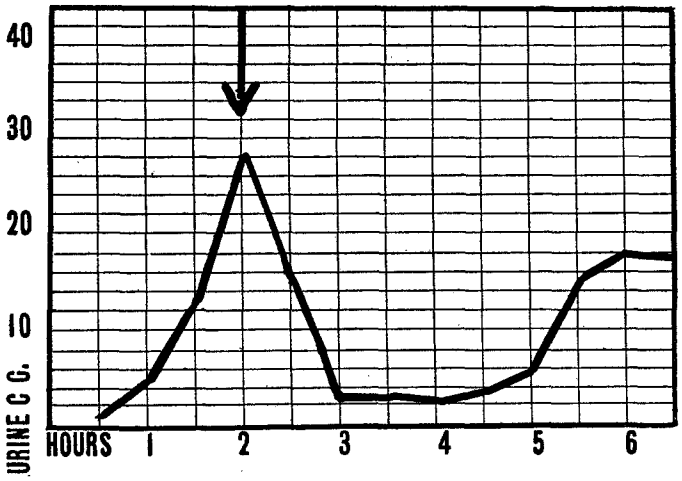


TEXT-FIG. 10. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates pituitrin, 1 cc. by mouth.

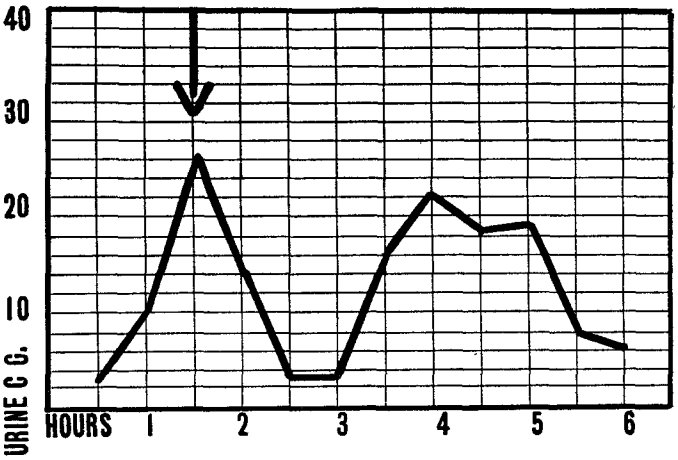
Subcutaneously.—(32 observations.) (Text-figs. 11, 12, and 13.) Here the onset is more abrupt, and with decreasing doses the duration of the effect is shorter. In small doses the effect is diminished also to the extent of the depression. These curves seem to indicate a limit of activity in dilution between 1:100,000 and 1:200,000, but the action is not constant beyond a dilution of 1:10,000. How this may be accounted for is unknown. On the other hand, the effect of large doses has been tried with the Parke, Davis preparation "400%." The action sets in rapidly, but even with this enormous dose, the output does not fall below 1 to 2 cc. in half an hour (Text-fig. 14). Apparently it is impossible to produce anuria in this way. The injections were well borne, except for cutaneous necrosis,

[1] Pituitrin "400%" is a special preparation (Parke, Davis and Company), which is four times the normal strength of pituitrin.

probably due to long lasting anemia of the skin, and the animals lived for a long time. Autopsy did not reveal pathological changes.



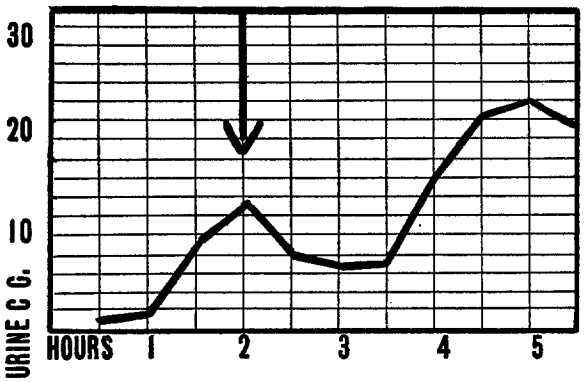
TEXT-FIG. 11. Rabbit, weight 1,600 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 800, 1 cc. subcutaneously.



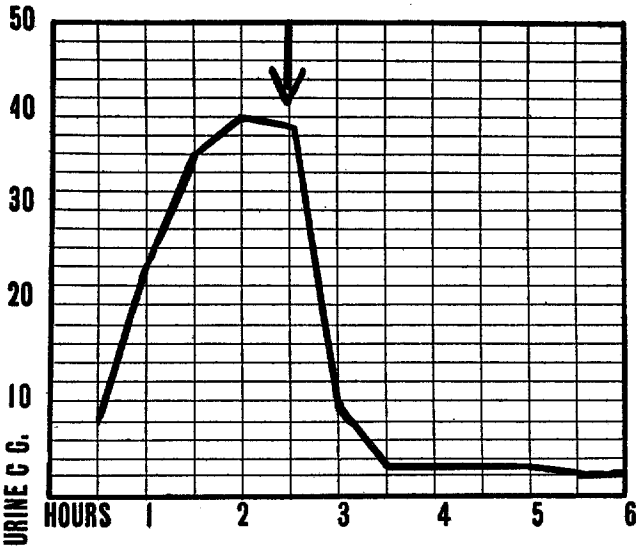
TEXT-FIG. 12. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 5,000, 1 cc. subcutaneously.

Intravenously.—(8 observations.) This mode of administration shows the same result of decreasing doses as to duration and degree

of depression (Text-figs. 15 and 16). Evidently the limit by this method is beyond 1 cc., dilution 1: 500,000 (Text-fig. 16), which



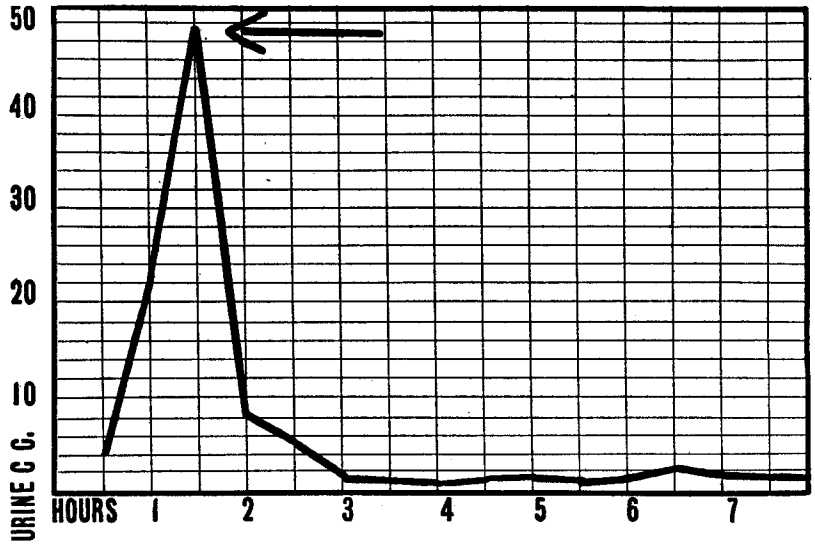
TEXT-FIG. 13. Rabbit, weight 2,200 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 100,000, 1 cc. subcutaneously.



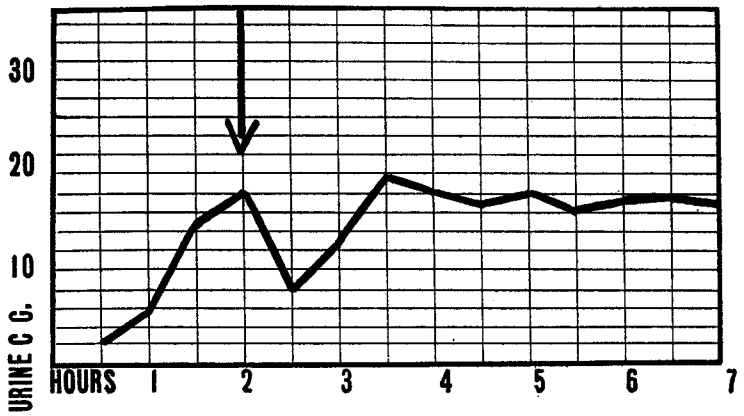
TEXT FIG. 14. Rabbit, weight 2,100 gm. Artificial polyuria. The arrow indicates pituitrin "400%," 12 cc. subcutaneously.

might possibly be of value as a biological test for the presence of pituitary secretion. My observations, however, are too few to warrant conclusions as to its reliability for this purpose. The effect

sets in more quickly, but in some instances there has been a latent period even by intravenous injections.



TEXT-FIG. 15. Rabbit, weight 2,800 gm. Artificial polyuria. The arrow indicates pituitrin, 2 cc. intravenously.



TEXT-FIG. 16. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates pituitrin, dilution 1: 500,000, 1 cc. intravenously.

As already mentioned, dogs are not suitable for experiments of this kind on account of the high concentration of their urine. I have,

however, made two observations on a dog in which polyuria had developed after partial pancreatectomy.[2]

Observation 1.—Dog; weight 13.2 kg. October, 1915. Partial pancreatectomy. Lowered carbohydrate tolerance. Sugar-free on meat diet. Diuresis averaged 200 to 300 cc. April, 1916, diuresis averaged 500 to 700 cc. (no sugar).

Pituitrin 1 Cc. Five Times Subcutaneously.

	Diuresis. cc.	*Sp. gr.*
Apr. 27	600	1,028
" 28	460	1,025
" 29	500	1,032
" 30	620	1,035
May 1	600	1,040
" 2	650	1,028
" 3	750	1,032
" 4	400	1,034
" 5	200	1,047
" 6	700	1,030

Observation 2.—The same dog.

Pituitrin 1 Cc. Three Times Subcutaneously.

	Diuresis. cc.	*Sp. gr.*
May 9	850	1,015
" 10	900	1,013
" 11	250	1,047
" 12	600	1,030
" 13	770	1,017
" 14	600	1,016
" 15	520	1,028

Is This Effect Dependent on Changes in General Blood Pressure?

A priori this seems unlikely, as it is agreed by most investigators that a subcutaneous injection of pituitrin exerts a slight influence or none on the blood pressure, and it would require a pronounced drop to account for the antidiuretic effect. Three observations in which the pressure has been registered for 3 hours after subcutaneous injection with pituitrin, have shown no change in pressure, while the output has been checked.

[2] I am indebted to Dr. J. Homans for the opportunity of making these observations.

To Which Part of the Pituitary Body Is This Action Due?

Watery extracts have been prepared from different parts of the same lot of bovine glands, made up in the same way as is pituitrin.

Anterior lobe extract (5 observations) gave a definite antidiuretic reaction in a dose of 1 cc., while 1 cc. of a dilution of 1:50 gave negative results.

The extracts from the pars intermedia (7 observations) and the posterior lobe (10 observations), however, had a marked antidiuretic influence in a dilution of 1:40,000 (1 cc. injected). The intermedia extract showed this effect most constantly, while the posterior lobe extract failed in some instances long before the dilution 1:40,000 was reached. The dilutions were not carried beyond 1:40,000, and subcutaneous injections were used exclusively.

Two other extracts from the anterior lobe (Parke, Davis and Company) also showed negative results when 1 cc. of a dilution of 1:50 was injected. These anterior lobe extracts have also produced a marked response on the guinea pig uterus (Dale apparatus).

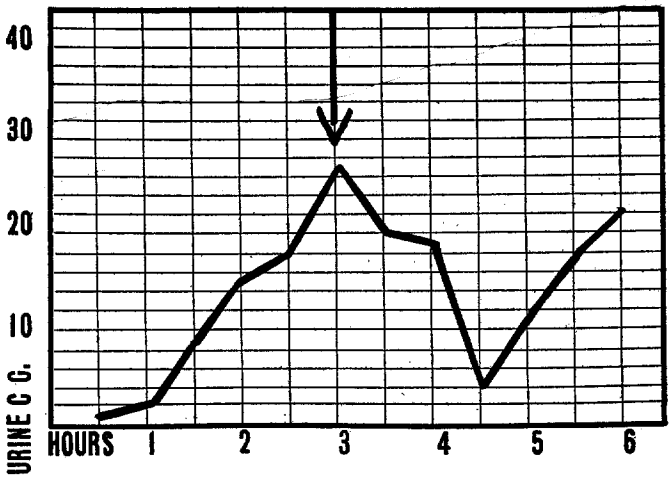
A special anterior lobe preparation, tethelin, which Robertson claims to be the growth-controlling principle of that lobe, has further been tested (4 observations). 1 cc. of a 5 per cent solution of this substance had a marked antidiuretic effect, and the limit of activity is between dilutions of 1:10 and 1:100.

It would thus appear justifiable to assume that the anterior lobe is relatively weak in antidiuretic action, while this power seems to be equally manifested by the pars intermedia and by the posterior lobe. These results are in agreement with clinical experience in as far as anterior lobe extracts are unable to check the output in diabetes insipidus.

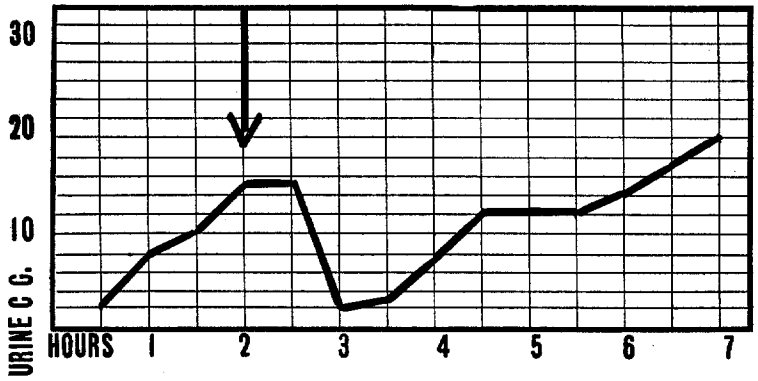
Active Principles of the Hypophysis.

In spite of the large amount of work done upon this subject, especially in the chemical laboratories of some of the leading drug firms, our knowledge is comparatively limited. Many compounds of doubtful specificity have been isolated, but although the term "active principle" is not yet justifiable, some of the bases isolated may be regarded at least in some way as functional equivalents. One of these compounds, β-imidazolylethylamine, is already used for the standardization of pituitary extracts.

Owing to the European war it has been very difficult to obtain these materials, and I have been able to secure small samples only of some of the compounds (Hoffmann La Roche).



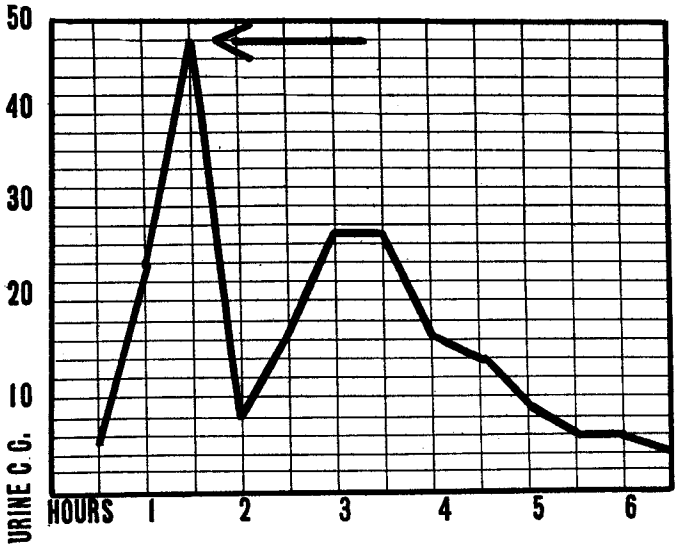
TEXT-FIG. 17. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine (Hoffmann La Roche), 20 mg. by mouth.



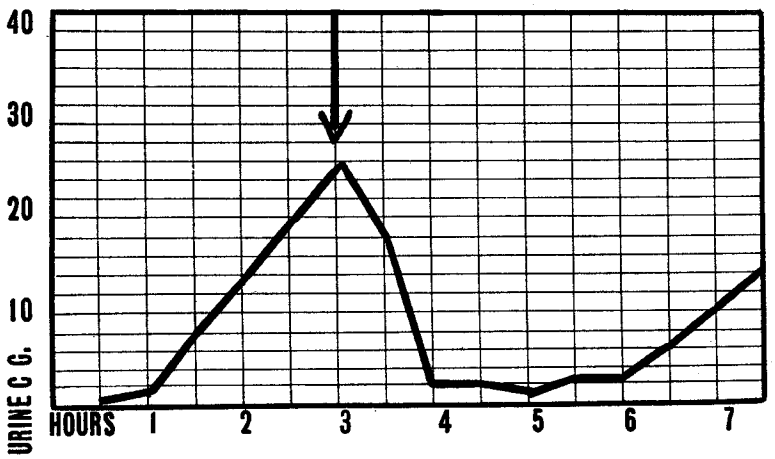
TEXT-FIG. 18. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine, 1 mg. subcutaneously.

β-Imidazolylethylamine. Orally.—(5 observations.) For 20 mg., the curves (Text-fig. 17) are in perfect agreement with the pituitary curves. 10 mg. had no such effect, and when exposed to air and light for 24 hours the solution had become inactive.

Subcutaneously.—(6 observations.) The curve for 1 mg. showed a positive result (Text-fig. 18), while 0.4 mg. only gave a slight antidiuretic response.



TEXT-FIG. 19. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates β-imidazolylethylamine, 1.5 mg. intravenously.

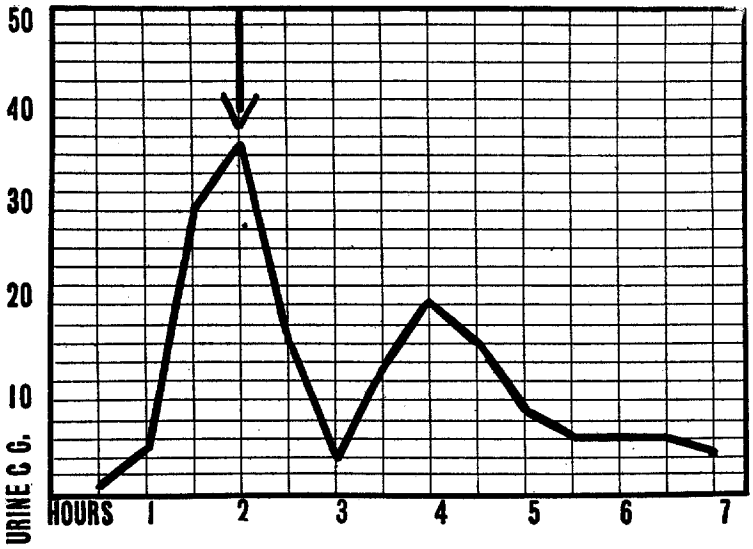


TEXT-FIG. 20. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates *p*-oxyphenylethylamine (Hoffmann La Roche), 10 mg. subcutaneously.

Intravenously.—(2 observations.) Strongly positive results were obtained for 1.5 mg. (Text-fig. 19).

p-Oxyphenylethylamine.—(1 observation.) Strongly positive results were obtained with 10 mg. subcutaneously injected (Text-fig. 20).

Experiments with the mother substances of these amines, histidine (1 observation) and phenylethylamine (1 observation), in 10 mg. doses, gave negative results. In this connection it will be of interest to see the effect of Secale (5 observations), the drug from which Barger and Dale first isolated β-imidazolylethylamine. A prepara-



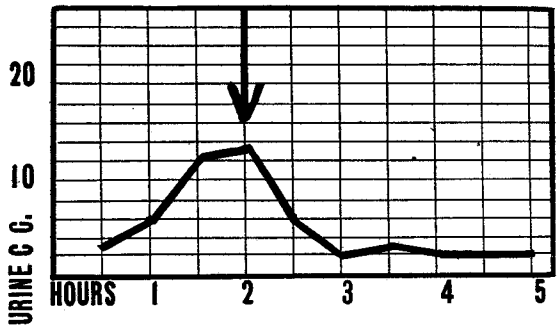
TEXT-FIG. 21. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates secacornin (Hoffmann La Roche), dilution 1: 10, 1 cc. subcutaneously.

tion from this drug gave positive results in a 1: 10 dilution (Text-fig. 21), while the reaction was almost negative in a dilution of 1: 50. This finding will naturally imply that the sympathetic nervous system or the blood vessels were involved in the action. Moreover, it is of interest because Secale is one of the drugs recommended in diabetes insipidus.

These results are suggestive, as the compounds belong to the series of amines which Barger and Dale have named "sympathomimetic

amines" on account of their general stimulating effect on the sympathetic nervous system. Unfortunately, it is at present impossible to procure in America other members of this highly interesting chemical group. Their antidiuretic action has not previously been demonstrated, but Dale and Laidlaw observed a decrease in the volume of the kidney following injection with β-imidazolylethylamine, which they believed due to active renal vasoconstriction. They found no effect on the secretion of urine,—probably on account of the short observation time.

Barbour and Quagliariello have been able to show that β-imidazolylethylamine constricts isolated arteries.



TEXT-FIG. 22. Rabbit, weight 1,500 gm. 0.7 per cent sodium chloride solution, 200 cc. subcutaneously. The arrow indicates pituitrin, 1 cc. subcutaneously.

Parenteral Water Administration.

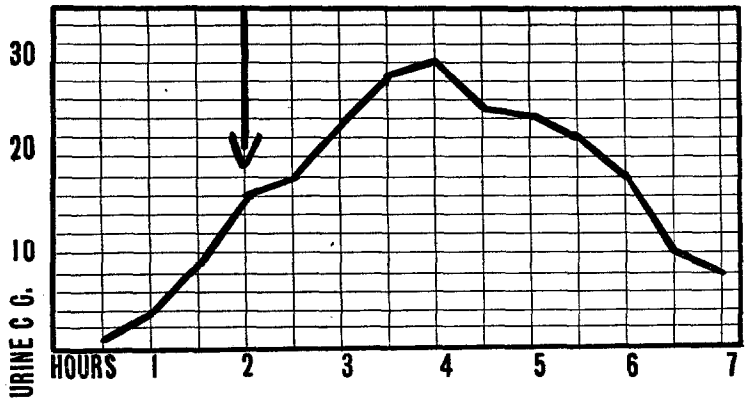
In order to rule out the remote possibility that the antidiuretic action might be due to influence on the intestinal absorption, some experiments have been performed in which polyuria was produced by saline solution, administered subcutaneously (3 observations). The pituitary extracts acted in the usual way (Text-fig. 22).

Influence of the Nervous System.

The marked influence of the nervous system upon the kidneys has been considered chiefly of vasomotor nature, the existence of true secretory nerves being as yet unproved.

Vagi.—(10 observations.) Opinions have differed considerably as to the influence of the vagi. Increase or decrease in output of urine has been described as the result both of stimulation and of division of the nerve. Some investigators, however, have been unable to demonstrate any influence.

The vagi were severed in the neck before beginning the experiments. This technique, however, proved unsatisfactory as the onset of polyuria was either delayed or did not occur, and I have reason to believe that this was due to gastric paralysis whereby the ingested water was not moved on. It was necessary, therefore, to postpone the division of the nerves until the water had left the stomach and poly-



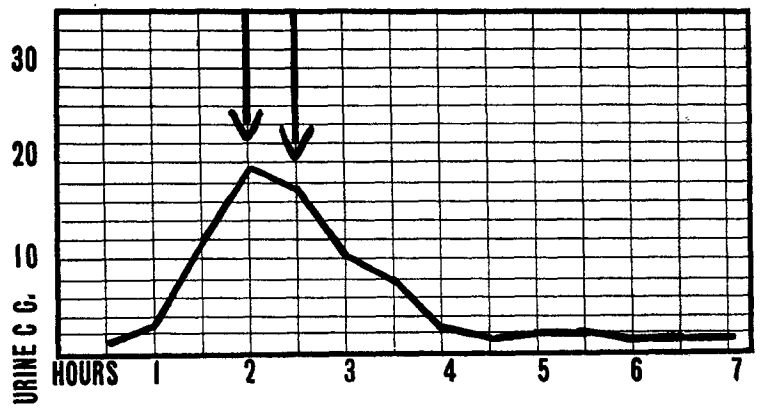
TEXT-FIG. 23. Rabbit, weight 2,000 gm. Artificial polyuria. The arrow indicates the division of the nervi vagi in the neck.

uria had set in. The operations were finished in 5 to 10 minutes and performed under ether narcosis. This procedure (Text-fig. 23) does not interfere with the normal course of the artificial polyuria, and gives a good test-object. The output, however, is checked in the usual way by pituitary injections (Text-fig. 24).

Splanchnic Nerves.—(8 observations.) Most investigators agree that stimulation tends to diminish the flow of urine. Division of the nerve is supposed to bring about polyuria in dogs but not in rabbits, though Jungmann and Meyer have observed polyuria of short duration also in these animals. The influence of the splanchnics is generally supposed to be dependent on vasomotor changes.

Section of both splanchnic nerves did not suit my purpose, as polyuria was not readily produced. This may be accounted for partly by the low blood pressure, which is apt to persist after the operation, and partly by the tendency of the animals to develop diarrhea upon water ingestion. Consequently only one splanchnic was cut and the kidney of the other side removed, this procedure being considered safe as several earlier investigators agree that the activity exerted by the splanchnic nerve on the kidney is strictly unilateral.

Splanchnicotomy in rabbits is a difficult operation and this undoubtedly accounts for the varying results. The nerve was exposed

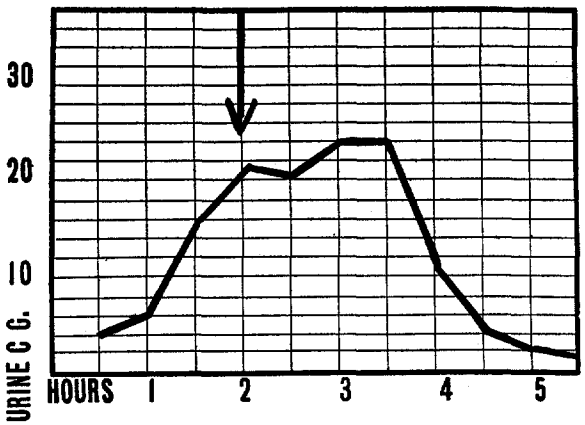


TEXT-FIG. 24. Rabbit, weight 1,400 gm. Artificial polyuria. The first arrow indicates the division of the nervi vagi. The second arrow indicates pituitary liquid, 1 cc. subcutaneously.

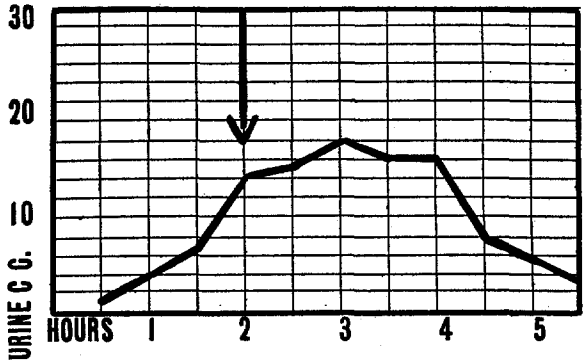
from the back in the retroperitoneal space and divided about 1 cm. above the adrenal, the procedure being performed under ether anesthesia. The nerve is small and the field narrow, and it is difficult, therefore, to be certain that all fibers have been cut. The observations were made 1 to 8 days after operation.

Some of the observations showed the usual response upon pituitary injection, but in others apparently no influence could be demonstrated (Text-figs. 25 and 26). Since, in my experience, 1 cc. of pituitary extract has never failed to check polyuria, I believe it justifiable, despite the limited number of experiments, to infer that the splanchnic nerve is the pathway for the normal antidiuretic action of

the pituitary extracts. If the curves do not justify the conclusion that the influence is entirely absent, it must at least be granted that the reaction is considerably delayed.



TEXT-FIG. 25. Rabbit, weight 1,600 gm. Division of the left splanchnic nerve and removal of the right kidney, 5 days before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

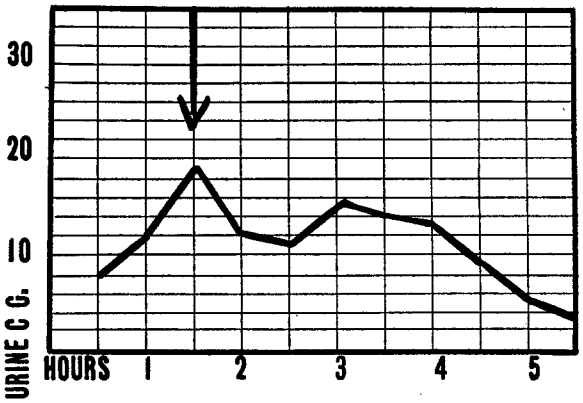


TEXT-FIG. 26. Rabbit, weight 2,000 gm. Division of the left splanchnic nerve and removal of the right kidney, 1 day before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

Renal Nerves.—(6 observations.) The kidneys of the rabbits were exposed transperitoneally, under anesthesia, through a median abdominal incision and the vessels carefully dissected near the hilus; the visible nerves were cut, and the outer layer of the artery partly

removed. As nerve fibers must still remain in the wall of the artery, it is impossible in this way to exclude nervous influence entirely, but approximately 90 per cent of the fibers are supposed to be divided. The observations were made 2 to 5 days after operation. Some of the animals gave the usual response to pituitrin while others acted in an unusual manner, the differences being possibly referable to variation in the number of functionally intact nerve fibers. As regards both degree and duration, some of the experiments show that the reaction is less pronounced than usual (Text-fig. 27).

The investigations of von Frankl-Hochwart and Fröhlich indicate that pituitrin acts as a stimulus to certain nerves belonging to the



TEXT-FIG. 27. Rabbit, weight 2,400 gm. Division of the renal nerves, 8 days before the observation. Artificial polyuria. The arrow indicates pituitrin, 1 cc. subcutaneously.

sympathetic as well as to the parasympathetic system, while the influence, according to Dale, is exerted directly on the involuntary muscles. As to the kidneys, however, this point has not been tested.

Is the Antidiuretic Influence Caused by Vasomotor Changes?

The nervous system has, as has been shown, a definite relation to this effect of the pituitary extracts, and the influence may be of vasomotor or secretory origin. As the existence of true secretory renal nerves remains doubtful, a foundation for discussion of this question is lacking. The general vasoconstrictor influence of pituitary ex-

tracts is, on the other hand, well known, but as to the renal vessels there is much confusion, apparently due to the methods employed.

Schäfer and his coworkers noted dilatation of the renal vessels, and Campbell, working on isolated renal vessels, confirmed this finding, while Dale found constriction of the isolated kidney. Pentimalli and Quercia, in perfusion experiments, observed decreased flow from the renal vein. Pal was able to demonstrate that strips from the outer layer relaxed, while the central parts contracted.

In dealing with these problems emphasis must be placed on the existence of peripheral vasomotor centers, the existence of which, in renal vessels, may be regarded as well established. It is, therefore, not justifiable to rule out nervous influence even after section of the nerves. It is possible, furthermore, that antidiuretic influence may be produced while the pituitary extracts are, at the same time, working on the nervous system and directly on the smooth muscles of the vessels.

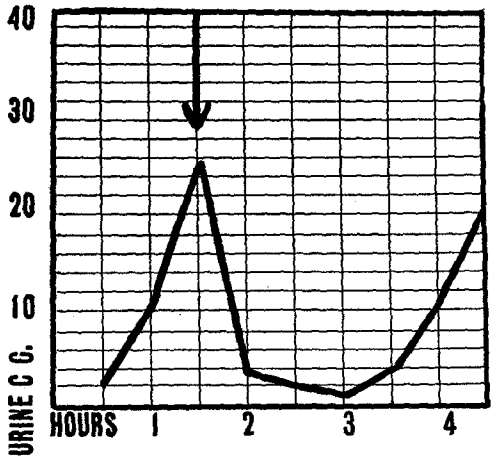
The vasoconstriction, which I believe is the chief cause of the checked output of urine, may be theoretically induced by stimulation of the vasoconstrictor or paralysis of the vasodilator nerves, but the assumption of an active vasoconstriction is the most likely explanation. With this assumption, the short antidiuretic response after dissection of the renal nerves may be accounted for by the fact that a part of the renal nerves between aorta and hilus is intact, and also by the remaining fibers in the wall of the vessels. Clinically, diabetes insipidus has for many years been considered to be due to vasomotor disturbances.

Pharmacological Studies.

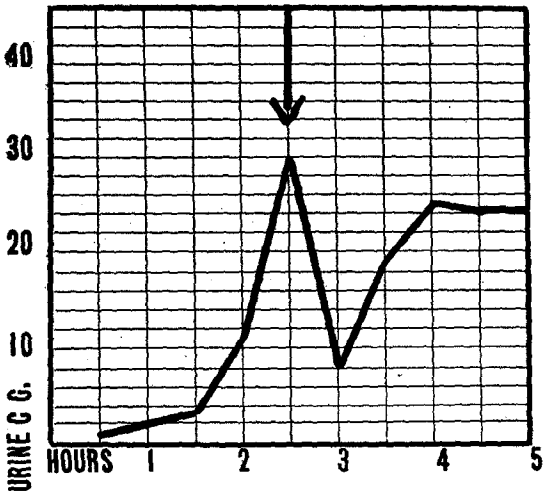
Nicotine.—(21 observations.) The influence of this drug shows a close similarity to the effect of the pituitary extracts (Text-fig. 28). The lower limit for the antidiuretic action of nicotine, which influence has not previously been demonstrated, is between 0.1 and 0.05 mg. intravenously injected into rabbits of medium size (Text-fig. 29). Subcutaneous injections with doses up to 30 mg. did not show such an effect. Two observations with blood pressure registration did not show changes in pressure during the time the output was checked.

Since the work of Langley and Dickinson, there has been a general

agreement that small intravenous doses of nicotine stimulate the sympathetic nervous system, and it therefore appears justifiable to



TEXT-FIG. 28. Rabbit, weight 2,900 gm. Artificial polyuria. The arrow indicates nicotine, 5 mg. intravenously.



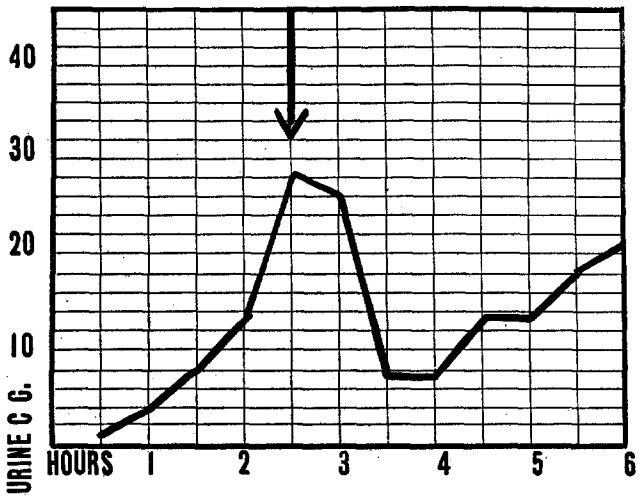
TEXT-FIG. 29. Rabbit, weight 1,500 gm. Artificial polyuria. The arrow indicates nicotine, 0.1 mg. intravenously.

refer the antidiuretic effect to such a stimulation. Judging, furthermore, from Langley and Dickinson's observation that nicotine,

painted on the renal plexus, produced constriction with subsequent dilatation of the renal vessels, it would seem that the antidiuretic power is exercised chiefly by means of these vessels.

I have not been able to block the sympathetic nervous system of the rabbit, as this requires large doses, 7 to 10 mg. per kilo intravenously being given as the smallest effective dose, and these are close to the fatal doses.

Caffeine.—(5 observations.) Subcutaneous injections with 0.15 gm. did not influence polyuria, while 0.20 and 0.25 gm. produced an inhibition of only 2 to 3 hours' duration (Text-fig. 30). This is prob-



TEXT-FIG. 30. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates caffeine, 0.25 gm. subcutaneously.

ably due to general vascular constriction, including the renal arteries, although toxic influence with lowered blood pressure could not be eliminated.

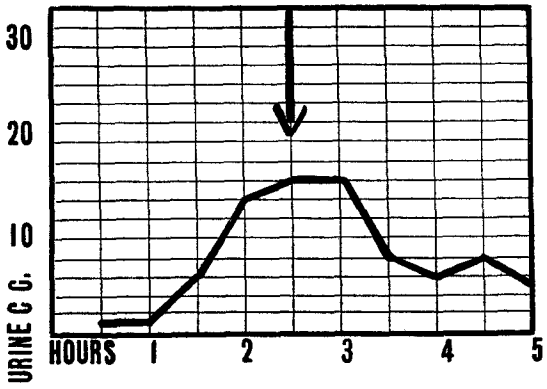
Strychnine.—(10 observations.) Subcutaneous injections with sulphate of strychnine, often in convulsive doses, from 0.06 to 0.54 mg., did not inhibit polyuria in rabbits weighing 1.5 to 2 kilos.

Strychnine is among the drugs usually recommended in diabetes insipidus.

Morphine.—(4 observations.) Subcutaneous injections with sul-

phate of morphine (8 to 65 mg. in rabbits weighing 1,500 to 1,900 gm.) did not show definite influence on the polyuria curves.

Chloral and Paraldehyde.—A series of experiments have been performed in which simultaneously with water ingestion chloral (10 observations) or paraldehyde (6 observations) has been administered orally. The doses have been chosen in accordance with what is given in the text-books as sufficient to paralyze the vasomotor nerves,—0.6 gm. of chloral per kilo, or 3 gm. of paraldehyde for a medium sized rabbit. These are the doses which are necessary to show the diuretic effect of caffeine, when permitted to act for 2 hours.



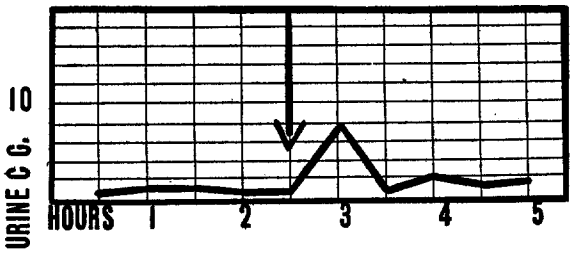
TEXT-FIG. 31. Rabbit, weight 1,300 gm. Artificial polyuria. Chloral 0.8 gm. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

I have been able to confirm the findings of earlier investigators that water diuresis is less readily produced under anesthesia, which fact is probably due to the low blood pressure. There are, however, serious objections against this method of blocking the vasomotor effect. The antidiuretic influence was very little changed under these circumstances (Text-fig. 31), except that the output in some instances did not go so low as after injection in normal animals. These observations disclosed another interesting fact; namely, that an inconstant initial diuretic effect occurred during the first 30 minutes (Text-fig. 32). This is no doubt the effect which has been found by Schäfer and most of the investigators who have worked with his method on anesthetized animals. This view may further be sup-

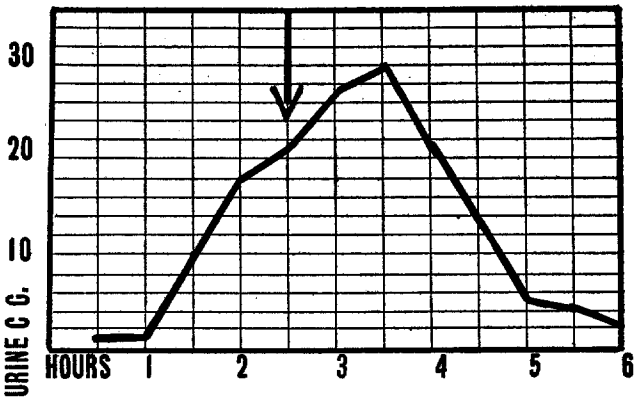
ported by Cow's general experience that when experimenting on unanesthetized animals the diuretic action of a substance may frequently be masked.

Influence of Other Ductless Glands.

Adrenal Medulla.—(28 observations.) Opinions have differed concerning the influence of adrenalin (Parke, Davis and Company)



TEXT-FIG. 32. Rabbit, weight 1,500 gm. Water, 20 cc. Chloral 0.9 gm. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

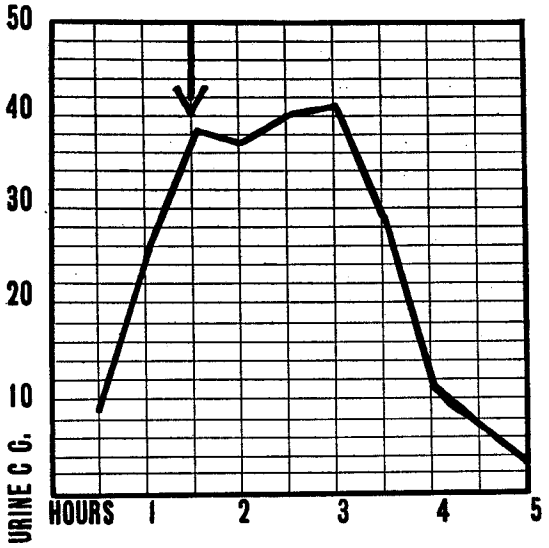


TEXT-FIG. 33. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates adrenalin, 2 mg. subcutaneously.

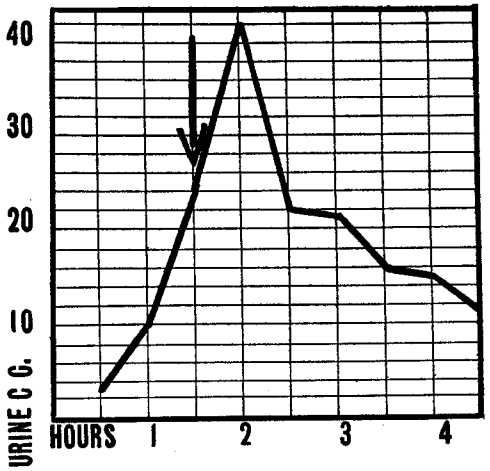
on diuresis. Most writers, however, agree that the general effect is a short, primary decrease and secondary increase in flow of urine. Judging from the knowledge that adrenalin has a constricting action also on the renal vessels and that pituitary extracts stimulate the adrenals to increased secretion, it would *a priori* appear reasonable to assume a cooperation between these glands as to antidiuretic effect.

Subcutaneously.—The polyuria is uninfluenced (Text-fig. 33).

Intravenously.—The appearance of the polyuria curve is not changed (Text-fig. 34).

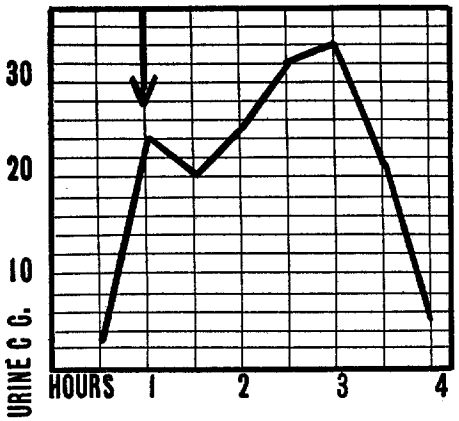


TEXT-FIG. 34. Rabbit, weight 1,700 gm. Artificial polyuria. The arrow indicates adrenalin, 0.01 gm. intravenously.



TEXT-FIG. 35. Rabbit, weight 3,000 gm. Artificial polyuria. The arrow indicates adrenalin, 0.05 mg. intravenously, every 3rd minute for 30 minutes.

Intravenously at Short Intervals.—As the effect of an intravenous injection with adrenalin is only of short duration, the drug has in some instances been administered intravenously every 3rd minute for about half an hour. In some instances there seems to be an increase in the output (Text-fig. 35), while other observations have shown a slight decrease (Text-fig. 36). An inhibition, however, corresponding to the effect of pituitary extracts has not been observed. Doses ranging from 0.001 to 2 mg. have been injected, and many of the animals have shown the usual signs of adrenalin intoxication at autopsy.



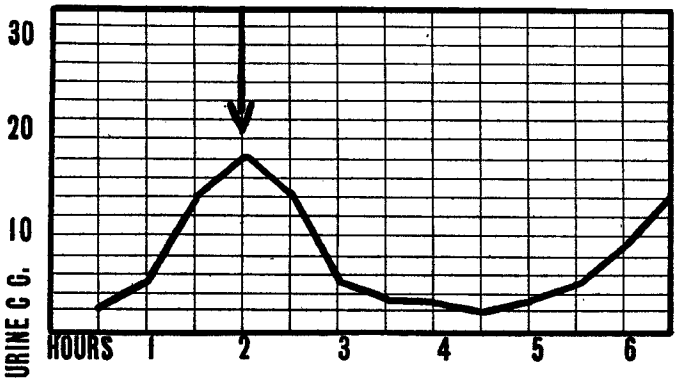
TEXT-FIG. 36. Rabbit, weight 2,900 gm. Artificial polyuria. The arrow indicates adrenalin, 0.05 mg. intravenously, every 3rd minute for 24 minutes.

In order to try the influence of other ductless glands, watery extracts have been prepared (Parke, Davis and Company) from different glands, made up in the same way as is pituitrin.

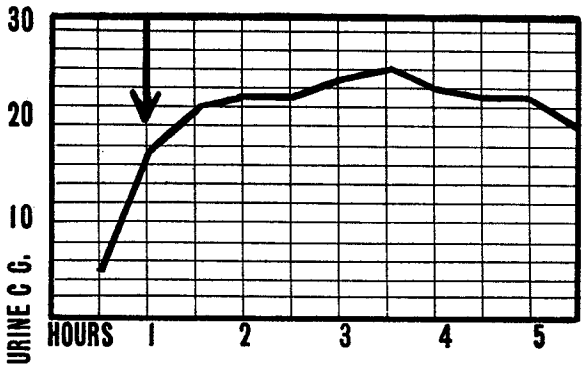
Adrenal Cortex.—(11 observations.) This showed a marked antidiuretic response, when administered subcutaneously in 1 and 2 cc. doses (Text-fig. 37), but the result was negative in a dilution of 1:10, and extract containing medulla as well as cortex did not possess this power in 1 cc. doses. The antidiuretic action of the adrenal cortex is in full harmony with the therapeutic results which Belfield obtained in diabetes insipidus by feeding the cortex. It is not likely, however, that the adrenals are concerned in the etiology of diabetes insipidus, judging from the fact that polyuria is not seen either in Addison's disease or after experimental removal of the adrenals.

Pineal Gland.—(6 observations.) Subcutaneous (Text-fig. 38) and intravenous injections both gave negative results.

There are a few cases of diabetes insipidus on record in which tumors of the pineal gland are held responsible for the polyuria (von



TEXT-FIG. 37. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates adrenal cortex extract, 2 cc. subcutaneously.



TEXT-FIG. 38. Rabbit, weight 1,800 gm. Artificial polyuria. The arrow indicates pineal extract, 1 cc. subcutaneously.

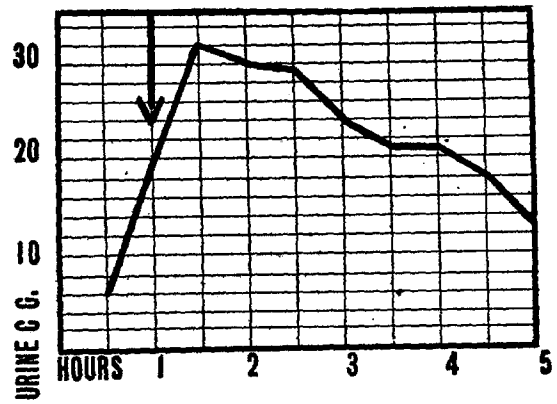
Gierke), but these cases may easily be referred to secondary influence on the pituitary body due to hydrocephalus.

Thyroid.—(6 observations.) Both subcutaneous (Text-fig. 39) and intravenous injections were negative.

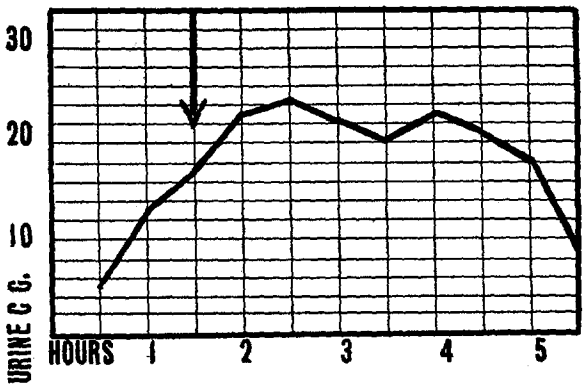
Thymus.—(7 observations.) The results were negative (Text-fig. 40).

Corpora lutea.—(5 observations.) These results were also negative (Text-fig. 41).

Pancreas.—(8 observations.) Injections gave negative results (Text-fig. 42) and no explanation is thus found for the polyuria com-



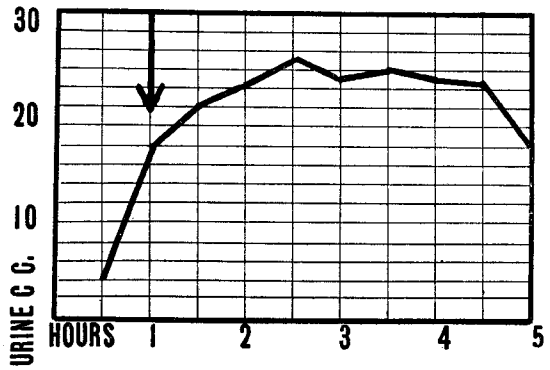
TEXT-FIG. 39. Rabbit, weight 1,900 gm. Artificial polyuria. The arrow indicates thyroid extract, 2 cc. subcutaneously.



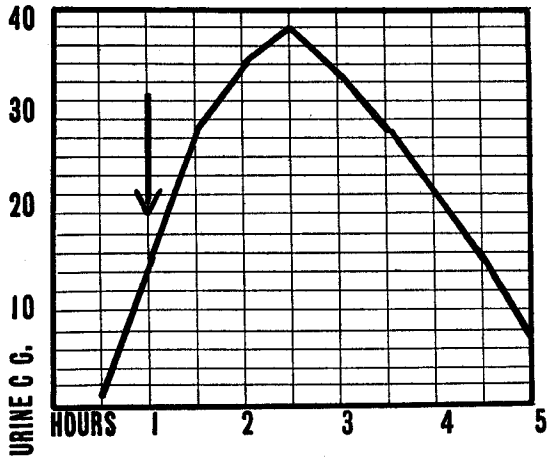
TEXT-FIG. 40. Rabbit, weight 2,600 gm. Artificial polyuria. The arrow indicates thymus extract, 1 cc. intravenously.

monly seen in dogs after partial pancreatectomy, or for the polyuria in diabetic patients.

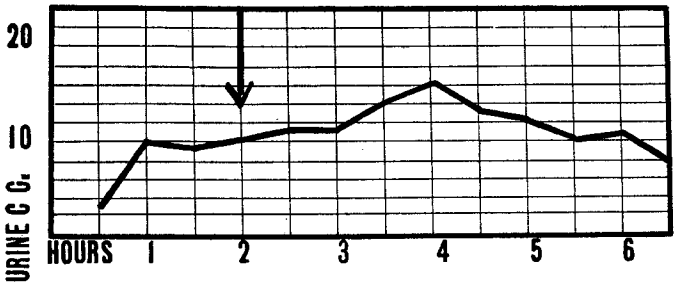
Ott and Scott have seen a diuretic effect from different ductless gland extracts, but the antidiuretic effect has not previously been tested.



TEXT-FIG. 41. Rabbit, weight 1,200 gm. Artificial polyuria. The arrow indicates corpora lutea extract, 1 cc. subcutaneously.



TEXT-FIG. 42. Rabbit, weight 2,100 gm. Artificial polyuria. The arrow indicates pancreas extract, 1 cc. intravenously.



TEXT-FIG. 43. Rabbit, weight 1,700 gm. 10 per cent sodium chloride solution, 40 cc. by mouth. The arrow indicates pituitrin, 1 cc. subcutaneously.

Influence on the Salt Diuresis.

Polyuria was produced for this purpose by ingestion by mouth of 40 cc. of a 10 per cent solution of sodium chloride (16 observations). The usual effective dose of pituitrin did not influence polyuria produced in this way (Text-fig. 43), or the influence was only slight. These results tend to demonstrate the fundamental differences between water diuresis and salt diuresis, and they show further the importance of a salt-poor diet in organotherapy of diabetes insipidus.

SUMMARY.

1. The inconstant results of past observations on the relation of pituitary extracts to renal activity have been due chiefly to unsuitable methods.

2. A standard curve of artificially induced polyuria may be plotted for rabbits, giving 200 cc. of water by mouth.

3. Extracts of the pars intermedia and posterior lobe of the hypophysis, given by mouth, subcutaneously, or intravenously, are able definitely to check polyuria thus induced. Extracts of the anterior lobe show a similar effect, but only to a slight degree.

4. This antidiuretic effect is constant, and is independent of (*a*) changes in blood pressure, (*b*) intestinal absorption, and (*c*) the vagi. The effect is apparently prevented or delayed by division of the splanchnics, and is diminished by division of the renal nerves near the hilus.

5. A similar antidiuretic property is possessed: (*a*) by β-imidazolylethylamine, (*b*) by *p*-oxyphenylethylamine, (*c*) by a preparation from *Secale cornutum*, (*d*) by small doses of nicotine, (*e*) by large doses of caffeine, and (*f*) by extracts of the adrenal cortex.

6. No effect on the polyuria is produced: (*a*) by strychnine, (*b*) by morphine, (*c*) by adrenalin, or by extracts of (*d*) thyroid, (*e*) thymus, (*f*) pineal, (*g*) pancreas, or (*h*) corpora lutea.

7. In animals under chloral or paraldehyde anesthesia a short and inconstant initial increase in flow of urine is seen.

8. The antidiuretic effect is absent or only slightly marked in checking the so called salt diuresis.

CONCLUSIONS.

These facts tend to suggest that the antidiuretic action exerted by pituitary extracts on rabbits is caused by stimulation of the sympathetic nervous system and that the renal vasomotor system in this respect is of chief importance.

Clinically these conceptions bring the polyurias related to disorders of the nervous system and the polyurias of pituitary origin in closer contact.

I wish to acknowledge the assistance of Dr. S. C. Harvey and Dr. W. C. Quinby in some of the operative procedures, the cooperation of Dr. J. Homans and Dr. T. Brailsford Robertson, and the courtesy of Hoffmann La Roche Company. I am indebted to Dr. Harvey Cushing for the privilege of carrying out this work in his laboratory under his direction.

I also wish to express special thanks to Dr. E. M. Houghton and Dr. C. P. McCord, of the biological laboratory of Parke, Davis and Company, for their help.

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